Micropropagation of *Lagerstroemia speciosa* (L.) Pers. (Lythraceae)

LIM-HO Chee Len and LEE Sing Kong
Botanic Gardens, Singapore

Abstract

Lagerstroemia speciosa (L) Pers., a common road-side tree of Singapore, was successfully mass-produced using the tissue culture technique. Nodal segments were the best explants as they could produce more multiple shoots than do shoot tips with BAP and 2ip treatments. The excised shoots were rooted in agar medium or sterilised sand, supplemented with IBA. The former was found superior. About 90% of the plantlets survived when transplanted to soil.

Introduction

Lagerstroemia speciosa is commonly planted along roadsides and in open spaces throughout Singapore for its shade. These trees also produce colourful flowers in abundance. There is some variation in the flowering characteristics of some of the trees in the population established. Some of them consistently flower more profusely than other trees located in close proximity. As it is the intention of the Parks and Recreation Department to introduce colours to our landscape effectively, it would be beneficial to plant avenues and groves of those trees recognised for their superior flowering qualities. A row of trees with uniform flowering habits is most desirable. To achieve this objective, there is a need to clone trees having the above characteristics. Although this could be achieved through rooted stem cuttings, the number that can be propagated is limited. Thus, the use of the tissue culture technique to mass propagate these trees is investigated and this paper reports the results of *in vitro* propagation of *Lagerstroemia speciosa.*

Materials and Methods

Young shoots from the crowns of mature trees as well as from the basal sprouts of tree stumps were collected. The leaves were removed and the axils were brushed lightly to remove dirt. This was carefully done so as not to break or damage the axillary buds at the nodes. The axillary buds were prominent and had an average length of 2-3 mm. Shoot tips and nodal segments with buds were separated, washed in tap water and used as explants. The length of the explants, i.e., shoot tips and nodal segments used were 5 mm and 10 mm respectively. They were then surface-sterilised by dipping in 98% ethanol for 5-10 seconds, and then in 30% clorox solution with Tween 80, for 45 minutes to 1 hour. The sterilised materials were rinsed five times in sterile water.

The basal medium consisted of Murashige and Skoog (1962) salt solution at the following concentrations: — a) macronutrient at half and, b) micronutrient at full strength, c) 3% sucrose. To test the response of explants, the media were further supplemented with IAA (Indole 3-acetic acid) and Kinetin (6 furfurylaminopurine). The concentrations used for both IAA and Kinetin ranged from 0-10.0 ppm, used either individually or in combination with one another. These concentrations were tested in 7 × 7 factorial combinations. There were 49 treatments altogether and each treatment was replicated 10 times.

For multiple shoot induction, media containing BAP (6-benzylaminopurine at 0, 1, 2, 3, 4 mg/l) or a combination of BAP at 1 mg/l and 2ip (N6 — $[\Delta^2$ isopentyl$]$ adenine at 1, 2, 3 mg/l) were used. Ten replicates of each treatment were prepared. After eight weeks of growth, shoot proliferation was evaluated in terms of increase in fresh weight, number of shoots formed and average length of shoots. The pH of the media was adjusted to 5.5 before autoclaving. The culture tubes were autoclaved at 120°C at 1.5 kg/cm^{-2} pressure for 20 minutes. The cultures were kept under 1 klx lighting provided by true-lite tube on a 12 hour photoperiod. The environment temperature was kept at 21-25°C.

Results and observations

The various explants responded differently when cultured on the various media. The pattern of response to the various treatments by each of the explants tested, are described below.

Growth of Nodal Segments and Shoot Tips

Table 1. Growth response of *Lagerstroemia speciosa* on MS salt with 49 combinations of IAA and Kinetin.

Kinetin mg/l IAA mg/l	0	0.5	1	2	2.5	5	10
0	C	—	—	—	—	CC	—
0.5	CC S	C S	C S	C S	S	CC S	CC
1	CC S	C S	C S	C S	S	CC S	CC
2	CC S	C S	C S	C S	C S	CC S	CC
2.5	—	—	—	—	—	—	—
5	—	CCC RR	CC RR	—	—	—	—
10	—	CCC RR	CC RR	—	—	—	—

Callus, shoot, root development were observed in 20-day old cultures.

C : slight callus
CC : vigorous growth of callus
CCC : abundant callus
S : shoot
— : no growth
RR : root

Of the 2 types of explants, nodal segments responded better and Kinetin had little effect on the growth (Table 1). Satisfactory callus and shoot formation were obtained with IAA at 0.5-2 mg/l. There was no significant difference in the response among the various combinations of Kinetin & IAA used. An average of 2 shoots developed from the axils of the nodal segment (Plate 1). Growth was first observed 7 days after inoculation, and about 20 days thereafter, shoots of 3-5 cm length were obtained. In most of the media, an initial swelling at the basal portion of the explant was observed. The swelling was due to cell divisions in the cortical layers leading to an increase of the tissue volume. After about 2 weeks, masses of friable and white callus were formed at the base of the culture shoots. Shoot tips on the other hand, usually browned off or showed less growth and they normally grew into single shoots (Plate 2).

Since nodal segments gave best growth, they were used as explants for all subsequent experiments.

Shoot multiplication

Multiple shoots developed within 15 days after inoculation. An average of about 3 buds were seen at each node and these developed into shoots in about 2 to 5 weeks. The best results were obtained in BAP medium at 1 mg/l (Table 2) and shoots reached a length of 1.8 cm. Increasing the BAP concentration promoted the growth of callus but caused a reduction in the shoot length (Table 2). The leaf blades were very much reduced, thus resulting in cane-like structures. There was no significant difference in the number of shoots formed.

Table 2. Effect of BAP on the induction of multiple shoots.

Concentration of BAP (mg/l)	Average no. of shoots *	Average shoot length
0	1	0.8 cm
1	3	1.8 cm
2	3	1.0 cm
3	1	0.7 cm
4	2	0.9 cm

* The shoots were counted in 30-day old cultures.

With the addition of 2ip into the medium, the multiplication of shoots improved markedly (Table 3). The average number of shoots increased three-fold to 9 shoots per explant. The average shoot length also increased from 1.8 to 3.2 cm and there was no significant difference at different concentrations of 2ip (1-3 mg/l). All the cultures showed healthy growth with a high frequency of normal leaves and shoots with long internodes (Plate 3). It was possible to mass-produce the shoots at this stage. Shoots which had elongated to about 5-10 nodes could be excised and cut into several nodal segments for further multiplication on the same medium. In these segments, the axillary buds enlarged and additional young shoots appeared in about 2 weeks (Plate 4). In about 6 weeks, they multiplied again (Plate 5) and could be excised for rooting. This process could be repeated to produce the required number of plantlets.

Table 3. Effect of combination of 1 mg/l BAP and 2ip (1-3 mg/l) on the induction of multiple shoots.

Concentration of 2ip (mg/l)	Average no. of shoots *	Average fresh weight of shoots	Average shoot length
1	9	4.2854 gm	3.05 cm
2	8.8	4.5852 gm	3.16 cm
3	9.2	4.5103 gm	2.99 cm

* The shoots were counted in 30-day old cultures.

Rooting of excised shoots

Tests were conducted on excised shoots of 3 to 4 cm length planted onto basal medium supplemented with IBA (Indole-3-butyric-acid at 0, 2, 3, 6 mg/l) or in sterile sand supplemented with MS salt solution and IBA (at 5, 10, 20, 40 and 60 mg/l). In each of the treatments, there were 10 replicates.

No root was formed in the control medium but with IBA added into the medium, healthy roots were formed (Plate 6). The average number of roots and the average root length did not vary with different IBA concentrations (Table 4). The most important effect of IBA was to induce roots and rooting percentage increased proportionally with the concentration until it reached 100% at 6 mg/l. At low IBA concentration, soft callus formed at the base of the tissues.

Table 4. Effect of different concentrations of IBA on rooting of shoots on MS agar medium.

Concentration of BAP (mg/l)	Average no. of roots *	Average length of root	Rooting Percentage
0	0	0	0
2	9.7	5.07	25
3	10.2	5.35	50
6	11.4	5.90	100

* The roots were counted in 30-day old cultures.

Table 5. Effect of different concentrations of IBA on rooting of shoots in sand medium, supplemented with MS salt.

Concentration of 2ip (mg/l)	Average no. of roots*	Average length of root	Rooting Percentage
0	9.8	1.04	20
5	15	1.2	21
10	25	1.15	50
20	20	2.1	42
40	20	1.9	32
60	19	1.5	35

* The roots were counted in 30-day old cultures.

For rooting in sand supplemented with different concentrations of IBA, the best results were obtained at the concentrations of 10-20 mg/l (Table 5). Although more roots were formed, they were short (Plate 7). Only about 50% of the cultures produced roots at the optimal IBA concentrations of 10-20 mg/l.

After rooting, the small plantlets were removed from the media and potted with either a mixture of coco-peat and sand (1:1) or a mixture of sand, coco-peat and soil (1:1:3). The potted plantlets were covered with a plastic sheet for about 2 weeks to maintain high humidity. Water stress is thus reduced and the development of epicuticular wax allowed (Grant & Aston, 1977). After 2 weeks to 1 month, the plants had hardened and the plastic sheets were removed. Ninety percent of the plantlets which rooted from agar media survived whereas only 50% of the plantlets rooted directly from sand survived. They all resembled plants produced by cuttings (Plate 8) and were similar in growth habit and form. However, whether rooted in agar media or sand, the potting-out survival rate was observed to decrease sharply for plantlets kept in the rooting culture for longer than two months.

Discussion

Tissue culture techniques provide viable alternative methods of mass-production of healthy plants with uniform characteristics. These techniques have been successfully applied to many ornamental plants such as orchids (Lim-Ho, 1982), herbs and shrubs (George & Sherrington, 1984). Although it is known that the explants of woody and tree species of Gymnosperms and Angiosperms are difficult to grow *in vitro* and to induce regeneration, methods for micropropagation of temperate and tropical trees have been developed from a variety of explant sources during the last few years (Bonga & Durzan, 1982; Dodds, 1983).

In this study we have demonstrated the feasibility of mass-production of L. *speciosa* using nodal segment and shoot tip cultures from stump sprouts. The ability of explants from stump sprouts to regenerate shoots *in vitro* is an advantage as plantlets produced would resemble the mother plants in terms of growth characteristics and habits. This would be especially useful when the main objective is to clone trees identified for their profuse flowering characteristics. The results of the present study also support the view that tissues at the basal region of a tree trunk still remain juvenile irrespective of the age of the tree (Bonga & Durzan, 1982; Vietiez et. al., 1983; 1985).

The most commonly used cytokinin for shoot multiplication in trees is BAP and its effectiveness used singly has been well demonstrated in the seedling tissues of *Calophyllum, Eugenia, Fagraea* (Lee & Rao 1980, 1982), *Swietenia* (Rao & Lee, 1982), and mature tissues of *Quercus robur* (Vietiz, 1985) and *Eucalyptus* (Gupta et. al., 1981) from *in vitro.* BAP alone was not effective in promoting shoot multiplication of *L. speciosa.* The addition of 2ip induced a higher multiplication rate, showing an interaction effect of BAP and 2ip on shoot multiplication. Such results are very rare in literature (Bonga & Durzan, 1982). Further studies could be initiated on the interaction effects of other cytokinins with BAP to gauge the response of shoot multiplication.

Rooting experiments in agar media were more successful than those tested on sand. Furthermore, when the plantlets were potted out, there was 90% survival rate. Further work would help to ascertain the best rooting media that would encourage better survival of plantlets when potted out. The present methods also show that it is possible to rapidly produce large numbers of plantlets of *Lagerstroemia in vitro* and it is suggested that this method is available for the propagation of other useful trees.

Acknowledgements

The authors wish to thank Mrs Quek-Phua Lek Kheng and Mrs Teo-Lee Guek Choon for their competent assistance in the experiments.

Literature Cited

Bonga, J.M. and D.J. Durzan (1982). *Tissue Culture in Forestry.* Martimus Nijhoff/ Dr. W. Junk Publications. The Hague.

Dodds, J.B. (1983). *Tissue Culture of Trees.* The Avi Publishing Comp. Inc. Weatport, Connecticut, USA.

George, E.F. and P.D. Sherrington (1984). *Plant Propagation by Tissue Culture.* Exegetics Ltd., Eversley, Basingstoke, England.

Grout, J.M. and D.C. Anston (1977). Transplanting of cauliflower plants regenerated from meristem culture, water loss and water transfer related to changes in leaf wax and xylem regeneration *Hort. Res.,* 17: 1-7.

Gupta, P.K., A.F. Mascarenhas and V. Jajannathan (1981). Clonal propagation of mature trees of *Eucalyptus citriodora* Hook, by tissue culture. *Plant Sci. Letter* 20: 195-201.

Lee, S.K. and A.N. Rao (1980). Tissue culture of certain tropical trees. In *Plant Cell Cultures:* Results and perspectives (Sala, F. and other Eds.). Elsevier/North Holland Biomedical Press. Amsterdam, 305-311.

______ & ______. (1982). *In-vitro* plantlet development in tropical trees — *Calophyllum inophyllum* and *Eugenia grandis. Tissue Culture of Economically Important Plants* (A.N. Rao ed.) COSTED Publ. Singapore 185-190.

Lim-Ho, C.L. (1982). Tissue culture of local orchid hybrids at the Singapore Botanic Gardens. *Tissue Culture of Economically Important Plants* (A.N. Rao ed.) COSTED Publ. Singapore 295-300.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco culture. *Plant Physiol.* 15: 473-497.

Rao, A.N. and S.K. Lee (1982). Importance of tissue culture in tree propagation — Proc. 5th Intl. Cong. *Plant Tissue & Cell Culture.* (Akio Fuji Wara ed.) Tokyo, Japan.

Vieitez, A.M., A. Ballester, M.L. Vieitez and E. Vieitez (1983). *In vitro* plantlet regeneration of mature chestnut — *Jour. Hort. Sci.* 58: 457-63.

Vieitez, A.M., M. Carmen San-Jose and E. Vieitez (1985). *In vitro* plantlet regeneration from juvenile and mature *Quercus robur*, L. *Jour. Hort. Sci.* 60: 99-106.

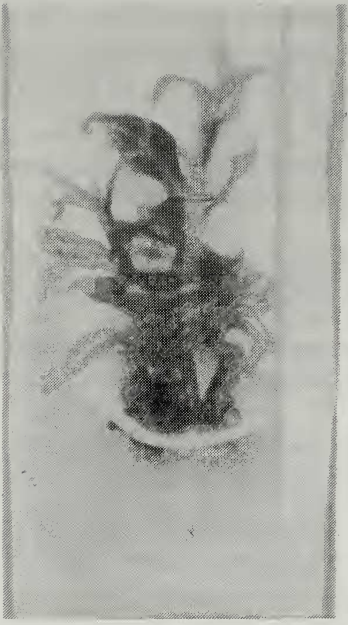

Plate 1. Two shoots developed from lateral bud (20-day old culture).

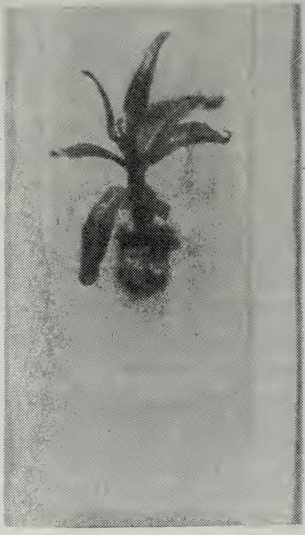

Plate 2. Single shoot developed from shoot-tip (20-day old culture).

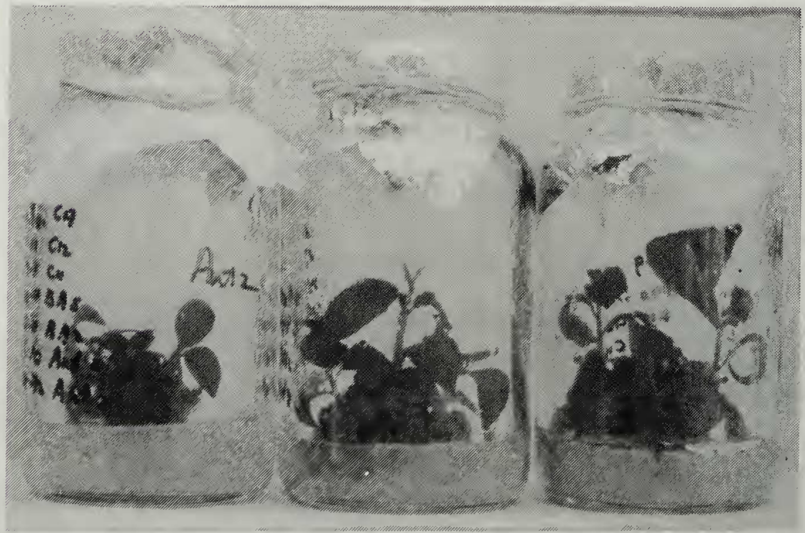

Plate 3. Multiple shoots on MS supplement with BAP (1 mg/l) + 2ip (1-3 mg/l) (18-day old culture).

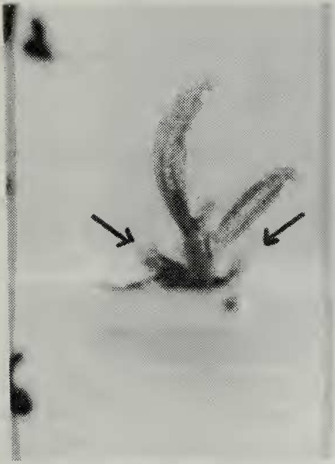

Plate 4. Young shoots arising from nodal segment (14-day old culture).

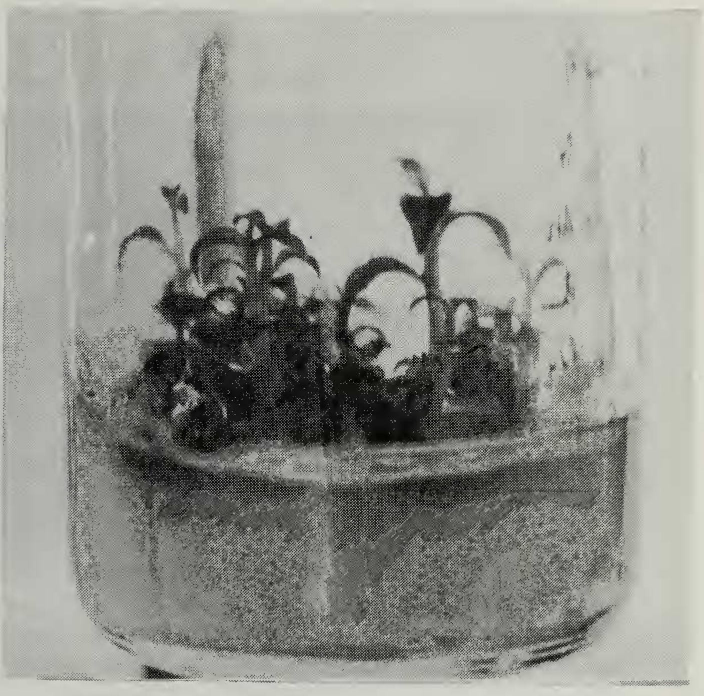

Plate 5. Multiple shoots from nodal segments (40-day old culture).

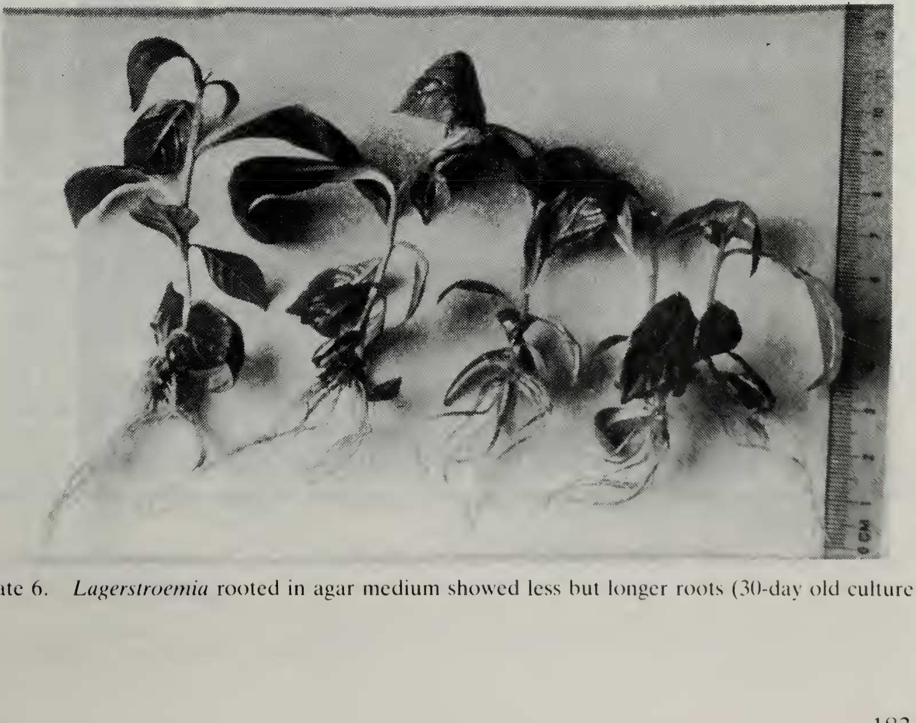

Plate 6. *Lagerstroemia* rooted in agar medium showed less but longer roots (30-day old culture).

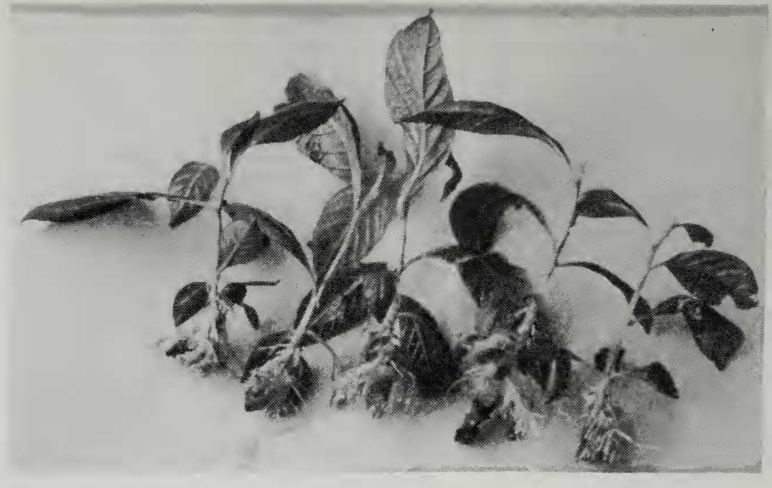

Plate 7. *Lagerstroemia* rooted in sand showed more but shorter roots (30-day old culture).

Plate 8. Well-established *Lagerstroemia* plantlets produced by tissue culture (60 days in planting media).